SINGLE NUCLEOTIDE POLYMORPHISMS IN GENE OF IL-1BETA IN BRONCHIAL ASTHMA

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ABSTRACT

Bronchial asthma is a common chronic lung disease that is driven by abnormal inflammatory reactions in the airways in response to the complex interaction between genetics and environmental factors. The underlying inflammation in asthma is manifested by prominent eosinophil infiltration and Th2 inflammatory mediators; however the pro-inflammatory cytokines, particularly IL-1β, have also been found in increased amount in the sputum and BAL in individuals with bronchial asthma, especially in more severe state. Therefore, IL-1β is considered to be of importance in pathogenesis of this condition as the protein level of the cytokine is finely regulated by variety of factors, including genetics. The IL1B gene displays many SNPs both in promoter and coding regions, which have been associated with IL-1β production.

In the current case-control study we investigated -511C>T (rs16944) promoter polymorphism and +3953C>T (rs1143634) silent polymorphism in exon 5 of IL1B and their haplotypes as candidate risk factors of Bronchial asthma in Bulgarian population.

We genotyped 47 patients with bronchial asthma and 174 control individuals using Taqman genotyping assay for IL1B -511C>T SNP and PCR-RFLP-based method for +3953C>T SNP. We did not observe statistically significant differences in genotype frequencies of IL1B -511C>T and IL1B +3953C>T between controls and patients with asthma (p=0.065 and p=0.987). However, the minor T allele of IL1B -511C>T was less frequently found in the controls (0.305) compared to the patients with asthma (0.415, p=0.0002). Carriers of IL1B -511T allele (TT or TC genotypes) appeared to have 2.25-fold higher risk for Bronchial asthma (95% CI: 1.127-4.498, p=0.019). The performed estimations of IL1B haplotypes did not reveal any difference in the haplotype frequencies between controls and patients with asthma (p=0.270). However, the T_C haplotype, constructed by alleles found to determine enhanced expression of IL-1β, appeared to be associated with higher risk of asthma (OR 1.78, 1.04-3.03, p=0.035) compared to the most common C_C haplotype.

Based on the results of the current study we suggest that the -511C>T promoter polymorphism and +3953C>T silent polymorphism in exon 5 of IL1B may influence the genetic predisposition of Bronchial asthma in Bulgarian population, as the carriers of alleles and haplotypes supposed to define higher IL-1β protein levels are more susceptible for this lung diseases.

Keywords: bronchial asthma, cytokines, polymorphisms, risk factors

Introduction

Bronchial asthma is one of the most common chronic lung diseases characterized by airway hyperresponsiveness, reversible airway obstruction and airway inflammation. The specific features of the inflammatory pattern in asthma are recruitment and activation in the airways of eosinophils, CD4+ lymphocytes, and mast cells and overproduction of bronchoconstrictor mediators, such as histamine, cysteinyl leukotrienes, kinins, and prostaglandin D2, as well as cytokines derived from Th2 cells (IL-4, IL-5 and IL-13) (23). Other key regulators are the eosinophilic chemotactic cytokine eotaxin and NO (2). In addition, the pro-inflammatory cytokines TNF-α, IL-1β and IL-6, known to be highly expressed in COPD, have also been found in increased amount in the sputum and BAL fluid in individuals with bronchial asthma, especially in more severe state (3, 4, 5).

Interleukin-1β (IL-1β) is considered as an important pro-inflammatory cytokine, primary produced by blood monocytes and tissue macrophages. IL-1β belongs to the IL-1 family of cytokines which consists of four main members: IL-1α, IL-1β, IL-1 receptor antagonists (IL1Ra) and IL-18 (6). Recently, a number of newly discovered molecules (IL-1HY1, IL-1HY2, FIL1ß, FIL1ε, FIL1η, FIL1ζ (7), IL-1H, IL-1RP1, IL-1RP2, and IL-1RP3) with clear homology to this group has expanded IL-1 cytokine family (7, 16, 18, 26). The genes for IL-1 family members, with the exception of IL18 (11q22.2-q22.3), are located in a cluster on the long arm of chromosome 2.
II-1 cytokines, particularly IL-1β, are major mediators of body’s response to bacterial infection, inflammation, immunological reaction and tissue injuries. The primary source of IL-1α and IL-1β are blood monocytes and tissue infiltrating macrophages, but they are also produced by many different cell types including neutrophils, dendritic cells (DCs), natural killer cells, B-lymphocytes, T-lymphocytes and cells from the central nervous system (10).

The IL-1 cytokines are inducible at transcriptional level by microbial products and host defense proteins, and the rate of expression has been proposed to be affected by the activity of promoter-enhancer regions of the corresponding genes. The principal II1 genes, including IL1B gene, have been found to displays variety of polymorphisms both in promoter and coding regions and these gene variants have been associated with the cytokine production (1, 8, 24, 25).

The IL1B gene displays many SNPs both in promoter and coding regions, which have been associated with IL-1β production. Among them are the IL1B -511C>T (rs16944) promoter polymorphism and the +3953C>T (rs1143634) silent polymorphism in exon 5 of IL1B (8, 9, 12, 21, 22, 24). No allele differences in nuclear protein binding were evident for -511C>T SNP, however the variant -511T allele has been shown to increase modestly the transcriptional activity when analyzed independently. In addition, when the -511T allele is in a haplotype with -31C allele of -31T>C SNP, a strong increase in promoter activity was found resulting in increase of IL-1β production (8). On the opposite, the more common +3953C allele of IL1B +3953C>T have been shown to elevate capacity to produce IL-1β in vitro (8).

To test whether promoter polymorphism of IL1B (IL1B -511C>T, rs16944) and the silent polymorphism in exon 5 of IL1B (+3953C>T, rs1143634) relate to Bronchial asthma, we conducted a genetic association study in a Bulgarian population.

Materials and Methods

Patients and control group

The patient group consisted of 47 individuals with Bronchial asthma aged from 19 to 85 years (median of 58 years). The control group consisted of 174 individuals, not affected by lung diseases, aged between 23 and 85 years (median of 61 years). The available demographic and clinical data are presented in Table 1.

Isolation of DNA

Genomic DNA was isolated from 0.2 ml whole blood samples using a commercial kit for isolation of genomic DNA from blood (GenElute™ Mammalian Genomic DNA Miniprep Kit, Sigma, USA).

Genotyping

The genotyping for IL1B +3953C>T was performed by PCR-RFLP-based method as it was described earlier (15). Amplification reactions were performed at a total volume of 12 µl using Eppendorf Mastercycler (Hamburg, Germany). Amplification mix contained about 30-50 ng of genomic DNA, 0.8 pmol/µl of each primer (IL1B-F: 5’-CTC AGG TGT CCT CGA AGA AAT CAA A-3’ and IL1B-R: 5’-TCT TTT TTG CTG TGA GTC CCG-3’), 200 µM dNTPs, 1x Buffer for Dream Taq Polymerase with 2 mM MgCl2 (Fermentas Life Science), 1U Dream Taq Polymerase (Fermentas Life Science) and ddH2O to the final volume. The amplification protocol was as the following: pre-amplification denaturation at 95°C for 3 min, 35 cycles of 30 sec at 94°C, 30 sec at 60°C and 30 sec at 72°C, followed by final extension at 72°C for 5 min.

Restriction reactions for IL1B +3953C>T SNP was performed in 16 µl final volume with 12 µl PCR reaction mixture and 3U Tag I for 16h at 65°C with. The fragments obtained after restriction reactions were analyzed after electrophoresis in a 3% agarose gel. The gels were stained with ethidium bromide and documented with EC3 Imaging System (UVP, UK).

The genotyping for IL1B -511C>T SNP was carried out by TaqMan genotyping assay. The PCR reaction mix consisted of 6 µl 2x TaqMan Genotyping Master Mix (Applied Biosystems, USA) 0.3 µl 40x SNP Genotyping assay with VIC® and FAM™-labeled probes (ID: C_1839943_10, Applied Biosystems, USA), 1.5 µl genomic DNA (about 20-30 ng/µl) and water to the final volume of 12 µl. The PCR profile was as the following: initial denaturation and activation of hot-start polymerase (95°C for 10 minutes) followed by 40 cycles (95°C for 15 sec and 60°C for 60 sec). Fluorescence data was captured using 7500 FAST Real-Time PCR System (Applied Biosystems, USA).

Statistical analyses

Statistical analyses were performed using StatView v.4.53, for Windows (Abacus Concepts, USA). The ANOVA test was applied for comparing the continuous variables in independent groups. The frequencies of distribution in contingency tables were analyzed using chi square test. The Odds ratios were calculated by using an interactive Online Software Package at the web site
TABLE 1
Demographic and clinical data of patients with Bronchial asthma and of the control individuals

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients with Bronchial asthma (N) (%)</th>
<th>Controls (N) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>males</td>
<td>13 (28%)</td>
<td>84 (48%)</td>
</tr>
<tr>
<td>females</td>
<td>34 (72%)</td>
<td>90 (52%)</td>
</tr>
<tr>
<td>Age at the inclusion in the study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean± SD (years)</td>
<td>54.19±17.8</td>
<td>59.93±11.1</td>
</tr>
<tr>
<td>median (range) (years)</td>
<td>58 (19-85)</td>
<td>61 (23-85)</td>
</tr>
<tr>
<td>Age at the diagnosis of the disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean± SD (years)</td>
<td>37.6±18</td>
<td></td>
</tr>
<tr>
<td>median (range) (years)</td>
<td>39 (2-72)</td>
<td></td>
</tr>
<tr>
<td>Duration of the disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean± SD (years)</td>
<td>15.6±13.8</td>
<td></td>
</tr>
<tr>
<td>median (range) (years)</td>
<td>12.5 (0-65)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-smokers</td>
<td>40 (85%)</td>
<td>76 (64%)</td>
</tr>
<tr>
<td>ex-smokers</td>
<td>4 (9%)</td>
<td>11 (9%)</td>
</tr>
<tr>
<td>current smokers</td>
<td>3 (6%)</td>
<td>32 (27%)</td>
</tr>
<tr>
<td>Smoking habits (packs/year)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean± SD, (range)</td>
<td>17.5±15.5 (5-40)</td>
<td>16.4±5.4 (10-25)</td>
</tr>
<tr>
<td>ex-smokers</td>
<td>25.0±13.2 (15-40)</td>
<td>16.8±12.3 (5-50)</td>
</tr>
<tr>
<td>current smokers</td>
<td>20.7±14.0 (5-50)</td>
<td>16.7±10.9 (5-50)</td>
</tr>
<tr>
<td>all smokers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1 % pr.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean± SD (range)</td>
<td>71.0±8.1 (60-79)</td>
<td>93.4±11.9 (82-113)</td>
</tr>
<tr>
<td>FEV1/FVC %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean± SD (range)</td>
<td>73.5±16.6 (55.8-99)</td>
<td>80.4±7.2 (76.3-93)</td>
</tr>
</tbody>
</table>

Results and Discussion
The PCR product amplified with the primers for IL1B +3953C>T SNP was of 194 bp in length. The Taq I digested the PCR product of the more common +3953C allele into 3 fragments with a length of 97bp, 85bp and 12 bp, whereas the PCR product of the +3953T allele is restricted into 2 fragments- 182 and 12 bp (Fig. 1).

We did not observe statistically significant differences in genotype frequencies of IL1B -511C>T and IL1B +3953C>T between controls and patients with bronchial asthma (p=0.065 and p=0.987), however the IL1B -511T allele was significantly less frequently found in controls (0.305) than in the patients with asthma (0.415, p=0.043) (Fig. 2A and Fig. 2B).
The logistic regression analysis defined that carriers of IL1B -511T allele (TT and TC genotypes) had 2.25-fold higher risk for development of bronchial asthma (95% CI: 1.127-4.498, p=0.019) (Table 2). Similarly, the IL1B -511T allele appeared to define 1.62-fold higher risk for asthma than the more common IL1B -511C allele (Table 2).

The performed estimations of IL1B haplotypes formed by the studied loci of IL1B (-511C>T and +3953C>T) did not reveal any difference in the haplotype frequencies between controls and patients with asthma (p=0.270, Fig. 3).

However, the T_C haplotype, formed by alleles -511T and +3953C, previously found to determine enhanced expression of IL-1β, appeared to be associated with significantly higher risk of asthma in the current study (OR 1.78, 1.04-3.03, p=0.035) compared to the most common C_C haplotype (Table 2).

In this study we examined the associations between two IL1B polymorphisms (-511C>T, rs16944, and +3953C>T, rs1143634) and Bronchial asthma. Our results indicate that these polymorphisms may influence the genetic predisposition to bronchial asthma in Bulgarian population, as the carriers of -511T allele, as well as T_C haplotype, formed by -511T and +3953C alleles, previously associated with higher IL-1β protein levels, are more susceptible for these chronic lung disease.
TABLE 2
Risk (OR) for development of Bronchial asthma according to the genotype and allele frequencies of IL1B -511C>T

<table>
<thead>
<tr>
<th>IL1B -511C&gt;T</th>
<th>Patients</th>
<th>Controls</th>
<th>OR (95% CI), p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Frequency</td>
<td>n</td>
</tr>
<tr>
<td>Genotype frequency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=47</td>
<td></td>
<td></td>
<td>n=174</td>
</tr>
<tr>
<td>CC</td>
<td>14</td>
<td>0.298</td>
<td>85</td>
</tr>
<tr>
<td>CT+TT</td>
<td>33</td>
<td>0.702</td>
<td>89</td>
</tr>
<tr>
<td>Allele frequency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-511C</td>
<td>55</td>
<td>0.585</td>
<td>242</td>
</tr>
<tr>
<td>-511T</td>
<td>39</td>
<td>0.415</td>
<td>106</td>
</tr>
</tbody>
</table>

TABLE 3
Risk (OR) for development of Bronchial asthma according to the haplotypes, constructed by IL1B -511C>T and IL1B +3953C>T SNPs

<table>
<thead>
<tr>
<th>IL1B -511C&gt;T and IL1B +3953C&gt;T</th>
<th>Patients</th>
<th>Controls</th>
<th>OR (95% CI), p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Frequency</td>
<td>n</td>
</tr>
<tr>
<td>Haplotype frequencies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=94</td>
<td></td>
<td></td>
<td>n=344</td>
</tr>
<tr>
<td>C_C</td>
<td>33</td>
<td>0.353</td>
<td>158</td>
</tr>
<tr>
<td>T_C</td>
<td>36</td>
<td>0.381</td>
<td>97</td>
</tr>
<tr>
<td>C_T</td>
<td>22</td>
<td>0.234</td>
<td>81</td>
</tr>
<tr>
<td>T_T</td>
<td>3</td>
<td>0.032</td>
<td>8</td>
</tr>
</tbody>
</table>

Bronchial asthma is a chronic inflammatory lung disease recognized as a T-helper type 2 (Th2) disease with a particular inflammatory pattern involving predominantly eosinophilia and Th2 mediators, such as IL-4, IL-5 and IL-13. Nevertheless, increased production of the pro-inflammatory cytokines, notably IL-1β in BAL (bronchoalveolar lavage), serum and bronchial biopsies has also been documented, especially in patients with more severe asthma (3, 4, 5, 17, 27). IL-1β has been originally reported as a blood monocytes and tissue macrophage product, but it is also produced by many different cell types including neutrophils, dendritic cells (DCs), natural killer cells, B-lymphocytes, T-lymphocytes and cells from the central nervous system (10). In asthma it has been suggested that mononuclear phagocytes can be activated through an immunoglobulin E (IgE)-specific mechanism to release pro-inflammatory cytokines like IL-1β (28) and the prompt production of pro-inflammatory cytokines, particularly IL-1β, was supposed to play a role in altering the airway responses in asthma (17, 29).
In this respect factors implicated in regulation of IL-1β cytokine production are of interest for evaluation of the risk of development and progression of asthma. Among them are the genetic polymorphisms, especially those located in regulatory regions, such as gene promoters, potentially affecting the transcriptional activity of gene (8, 9, 12, 21, 22, 24).

So far in the scientific literature there is quite limited number of studies focusing on the possible involvement of IL1B SNPs in bronchial asthma (11, 13, 14, 20, 31, 32). In most of the aforementioned works IL1B -511C>T SNP was studied, and predominantly no difference in the genotype and allele frequencies has been observed between asthmatic patients and controls (11, 14, 20). The silent polymorphism in exon 5 (IL1B +3953C>T) did not also reveal any significant association with bronchial asthma when studied in a population of Taiwan (11). However, the IL1B -511T allele was associated with asthma in Finish men (14) and in Turkish children (32).

Conclusions
Thus, the results of the latter two reports, as well as our current preliminary case-control study, describing higher frequency of "high-producing" alleles and haplotypes of the functional IL1B SNPs (-511C>T and +3953C>T) in asthmatic patients, taken together may suggest a role of these polymorphisms as predisposing factors for asthma and may provide some insights into mechanisms of pathogenesis of asthma. However, taking into account the complex nature of asthma, the effects of other genetic factors as well as the effects of environmental factors should be acknowledged in order to clarify the intima mechanisms of asthma development.

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REFERENCES